International Journal of PharmaO2
Journal Home Page: http://www.ijpo.in/
(IJPO: A Peer-reviewed Bi-monthly online journal)

Research Article

Mupirocin Loaded Microemulsion Based Gel for Effective Treatment of Burns

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ARTICLE INFO

Article history:
Received: 13/07/2021;
Revised: 30/07/2021
Accepted: 02/08/2021;
Available online: 01/09/2021.

Key Words:
Burns, lavender oil, microemulsion, mupoirocin, zeta potential.

Please cite this article as:
Dhurke, R. *et al*. Mupirocin Loaded Microemulsion Based Gel for Effective Treatment of Burns. 3(5), 0199- 0209.

ABSTRACT

The main objective of the research work was to develop microemulsion based gel of mupirocin in order to achieve a better permeation rate and high drug retention into the deeper layers of the skin for effective treatment of burns. Mupirocin is a topical antibiotic isolated from *pseudomonas fluorescens* which is effective against Gram positive bacteria. Saturation solubility studies were conducted and pseudo-ternary phase diagrams were constructed for microemulsion formulations of lavender oil, rose oil and tea tree oil using tween 80 and ethanol as surfactant and co-surfactant respectively. Developed microemulsions were characterized for various parameters such as pH, viscosity, thermodynamic stability studies, *in vitro, ex vivo* diffusion and drug retention studies. From pseudo-ternary phase diagrams formulation LMF8 was considered to be best and was incorporated into a gel base. Mupirocin loaded microemulsion gel showed drug permeation of more than 93 % and 63% for *in vitro* and ex *vivo* studies conducted for 6 h. The drug retention in dermis was found to be 46.4% for LMG8 while controlled gel showed drug retention of only 8.9 % through porcine ear skin. This indicated that mupirocin loaded microemulsions showed effective retention of drug in skin layers. Zeta potential was found to be - 43.5 with a polydispersity index of 0.171 which indicate good stability of microemulsion formulation.

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INTRODUCTION

Microemulsion systems are being increasingly investigated for transdermal, ocular, nasal, pulmonary, vaginal, rectal and intravenous drug delivery (Talegaonkar, *et al* 2008). A lot of study and research have been done over microemulsions as a potential drug delivery system (Senthil, *et al* 2011, Kantaria, et al 2003). Microemulsions have very low surface tension and small droplet size which results in high absorption and permeation. The characteristics such as increased drug solubilization, better thermodynamic stability and the ease of manufacturing gives microemulsions the edge

over the other formulations (Tenjarla, 1999, Constantinides, 1995).

Mupirocin is an antibiotic isolated from Pseudomonas fluorescens and is structurally unrelated to any other antibiotics. It is a medium potency, synthetic, non-fluorinatedantibiotic used topically and primarily effective against gram positive bacteria. It is bacteriostatic at low concentrations and bactericidal at high concentrations. It can be used for the treatment of burns, furuncles, impetigo and open wounds (Strock*et. al*, 1990, Ward, *et al*, 1986).

A burn is a type of injury to skin , or other tissues, caused by heat, electricity, friction, or radiation and corrosive chemicals that denature the proteins in the skin cells (Herndon 2002)Burns are characterized by severe skin damage in which many of the affected cells die. Depending on the cause and degree of injury, most people can recover from burns without serious health consequences. More serious burns require immediate emergency medical care to prevent complications and death.There are several topical formulations available for the treatment of burns like ointments, solutions, conventional gels, creams, foams etc., Topical delivery system refers to application of a drug on the surface of the skin to deliver the drug to the skin to treat dermal disorders. Microemulsion has emerged as an excellent tool for delivering poor water soluble drug to the deeper layer of skin with a view to decrease their adverse effects via decrease in dose. The present research work aims in developing mupirocin loaded microemulsions for effective treatment of burns.

MATERIALS AND METHODS

Materials

Mupirocin was procured as a gift sample from Cipla Ltd (Mumbai, India). Carbopol 934 was a kind gift from Lubrizol Advanced Materials India Pvt. Ltd, Tween 80, Propylene glycol, Isopropyl myristate, polyethyleneglycol 400 and ethanol were purchased from S. D. Fine Chemicals Ltd, Mumbai, India. Rose oil, lavender oil and tea tree oil, olive oil, basil oil, peppermint oil, argon oil were purchased from Sreeji Aroma Mumbai, India. All other reagents used were of analytical grade, double distilled water was used throughout the studies.

Methods

Saturation solubility studies

The saturation solubility studies of mupirocin were carried out in different solvents (Ramesh *et al*; 2010) and buffers such as distilled water, methanol, ethanol, oleic acid, poly ethylene glycol 400

(PEG 400), propylene glycol (PG), span 80, tween 80, peppermint oil, teatree oil, lavender oil, rose oil, rosemary oil, pH 6.4 phosphate buffer, saturated solutions of mupirocin were prepared by adding excess amount of drug to each selected vehicle and were agitated on the shaker for 48 h at 25º C. After reaching equilibrium, samples were collected and centrifuged at 10,000 rpm for 15 min. Further 100 µL of supernatant was collected and suitably diluted with methanol and mupirocin was quantified by using UV-Visible spectrophotometry at 225 nm.

Construction of pseudo ternary phase diagram

The pseudo-ternary phase diagrams were constructed by instillation of homogeneous liquid mixtures of oil, surfactant and cosurfactant with water at ambient temperature (Djordevicet al.2004). Rose oil, lavender oil, teatree oil were selected as oily phases, tween 80 as surfactant and ethanol as cosurfactant based on their solubility. For each pseudo-ternary phase diagram oil and surfactant to co-surfactant (S_{mix}) mixtures were prepared at weight ratios (w/w) of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9 (Ramesh, *et al*., 2010). Double distilled water was added drop by drop to each oily-surfactant mixture under magnetic stirring until the mixture became clear at a certain point. The concentrations of the components were recorded in order to complete the pseudo-ternary phase diagrams, and the contents of oil, surfactant, co-surfactant and water at appropriate weight ratios were selected based on the transparency and stability of formed microemulsions.

Development of microemulsion formulations

The concentrations of oil, water, surfactant and co-surfactant were varied in each case keeping the concentration of drug constant. Predetermined amount ofdrug was accurately

weighed and dissolved in oil. Co-surfactant and surfactant were added tooily solution of the drug and mechanically stirred (Magnetic stirrer, Remi Equipment's Pvt. Ltd.)to form an emulsion. Water was added drop-wise to the emulsion till the formation of tatransparent solution which indicated formation of microemulsion.Based on the pseudo-ternary phase diagrams oil phase and Smix was selected for the development of formulations as shown in table 1.

Characterization of Microemulsions

Measurement of pH

The pH values of the microemulsion samples were measured by pH meter (Remi equipment Pvt Ltd). The pH meter was calibrated before each use with buffer solution of pH 4.0, 7.0 and 9.0. The measurement of pH of the formulation was done in triplicate and mean values were calculated. (Bhajpai, M. *et al.*,2009)

Measurement of viscosity

The viscosities of microemulsions were measured with a Brookfield viscometer DV–II+ PRO, equipped with spindle no.LV1 has spindle code as 61.

Determination of drug content

Drug content was estimated by placing one dose equivalent microemulsion formulation in a 100 mL volumetric flask, to it add small amount of methanol which solubilizing the microemulsion finally make up the volume to 100 mL with pH 6.4 phosphate buffer. Shake well for about 1h in a shaker followed by centrifugation then analyzed. The resultant solution was filtered through Whatman filter paper and absorbance was measured at 225 nm using UV-Visible spectrophotometer.

Measurement of droplet size and zeta potential

The mean droplet size and zeta potential was determined by photon correlation spectroscopy using zeta sizer (Malvern instruments, UK). Each sample was diluted to a suitable concentration with filtered double distilled water. Globule size analysis was performed at 25°C with an angle of detection of 90°C. Size and poly dispersity index of microemulsions were obtained directly from the instrument.

***In vitro* drug diffusion studies**

In vitro drug diffusion studies were conducted for thermodynamically stable microemulsions by using franz-diffusion cell with an effective diffusion area of 2 cm^2fitted with a modified dialysis membrane. The receptor compartment was filled with 18mL of phosphate buffer (pH 6.4) and a small bar magnet was used to stir the elution medium at a speed of 600 rpm with the help of magnetic stirrer. The temperature of the elution medium was maintained and controlled at 37±1°C by a thermo static arrangement to mimic *in vivo* condition. An aliquot of 1 mL was withdrawn at a predetermined time interval replaced by an equal volume of elution medium to maintain sink conditions, diffusion studies were carried out for a period of 6 hours. The drug concentration in the aliquot was determined by UV-Visible spectrophotometer at 225 nm by using the standard curve. Amount of drug diffused at a various time intervals was calculated and plotted against time for all the developed formulations.

Thermodynamic Stability Studies of Microemulsion

The Prepared microemulsion formulation was evaluated for physical thermodynamic stability tests by following process:

Heating cooling cycle- 6 cycles between refrigerator temperature 4°C and 45°C with storage at each temperature of not less than 48 hours were studied. Those microemulsions which were stable at these temperatures were subjected to centrifugation test.

Centrifugation- Emulsions of respective formulations which passed heating and cooling cycle were centrifuged at 3500 rpm for 15 min. Those microemulsions that did not show any phase separation were taken for freeze thaw stress test.

Freeze thaw cycle-Three freeze thaw cycles between -21 ^{0}C and +25 ^{0}C with storage at each temperature for not less than 48 hours was done for the microemulsions.

Dilution test- When microemulsions are diluted with excess water, standard phosphate buffer (pH 6.8), 0.1 N HCl, and stored for 12h should not show any signs of precipitation or phase separation.

Formulation Development of Microemulsion Based Gel

The best microemulsion formulation of lavender oil was incorporated into gel base for further study. About 1g of carbopol 934 was selected as gelling agent and soaked in the 100 mL of distilled water overnight. The formed gel base was neutralized by drop wise addition of tri-ethanolamine (TEA) till the pH was adjusted to 6.4. To the gel base mupirocin loaded lavender oil based microemulsion equivalent to 20 mg dose was dispersed slowly with the help of overhead stirrer.

Characterization of Microemulsion Based Gel

Physical appearance of gel

The prepared microemulsion based gel (MEG) of lavender oil and drug solution were inspected visually for their colour, appearance and consistency.

pH measurement

The pH of the MEG was measured on digital pH meter (Remi equipment Pvt. Ltd) at ambient temperature. Before the measurement pH meter was standardized using pH 4.0 and 7.0 standard buffers and triplicates were done.

Rheological study

The rheological analysis of the MEG was performed using a Brookfield viscometer DV–II+ PRO, equipped with standard spindle LV3 with spindle code 63. Viscosity was done in triplicates and the mean value was calculated at 100 rpm.

Drug content

For determination of drug content, about 1 g of the microemulsion based gel which was equivalent to 10 mg was weighed and dissolved in methanol and the volume was made up to 100 mL. The above solution was diluted appropriately and drug content was determined spectrophotometrically at 225 nm.

Spreadability

Two glass slides were taken and onto one slide an excess of 3g of gel was placed. Then another glass slide was placed such that gel was sandwiched between two glass slides. The top slide was subjected to a stress of 50 gm by putting weight on it. Time (in sec) required by the gel to travel a distance of 10 cm was noted. A shorter time interval indicates better spreadability (mutimer, *et al*,1956).

***In vitro* drug diffusion study from dialysis membrane**

An *in vitro* drug release study was performed using franz diffusion cell shown in figure 2 Dialysis Membrane (Hi Media, molecular weight 5000 Daltons) was placed between receptor and donor compartments. Microemulsion based gel equivalent to 20 mg in 1g gel was placed in the donor compartment and the receptor compartment was filled with pH 6.4 phosphate buffer (18 mL). The diffusion cells were maintained at 37 ± 0.5° C with stirring at 600 rpm (Remi, India) throughout the experiment. At fixed time interval, 1 mL of aliquots were withdrawn for every 30 min, 1, 2, 3, 4, 5, and 6h from receiver compartment through side tube and equal aliquots were replaced. The samples were analyzed by UV- Visible spectrophotometry at 225 nm. Drug flux (µg/hr/cm^2) at steady state was calculated by dividing the slope of the steady state portion of the line in the plot of drug amount permeated per unit area of dialysis membrane verses time.

***Ex vivo* skin permeation studies**

Ex vivo skin permeation study was carried out in franz diffusion cell using porcine ear skin.

Preparation of skin-The skin of porcine ear was trimmed and adhering subcutaneous fat was cleaned carefully. Now the excised skin was clamped between the donor compartment facing stratum corneum and dermis facing the receptor compartment of franz diffusion cell. Then, 1 g of microemulsion based gel containing 20 mg of drug was applied on the donor compartment. The receptor compartment was filled with pH 6.4 phosphate buffer and maintained at 37°C with stirring at 600 rpm. At predetermined time intervals, 1 ml receptor medium was withdrawn through side tube for different time intervals up to 6h and the same volume of pure medium was immediately added into the receptor compartment. Th. All samples were filtered through Whatman filter paper and analyzed by UV spectrophotometer at 225 nm.

Estimation of drug retained in the skin layers- The skin was removed from the diffusion cells after completion of experiments. The surface of

skin specimens was washed 10 times with 1 mL distilled water and the drug content in the washings was determined using UV spectrophotometer. By the heat method epidermis and dermis layers were effectively separated. The skin specimen was placed in a sealed bag and placed in water maintained at 52º C for 30 seconds. After 30 seconds the dermis and epidermis were separated by peeling. The separated dermis and epidermis layers are minced with a surgical sterile scalpel and placed in 10 mL methanol and vortexed for 5min. The tissue suspensions were then centrifuged at 10,000 rpm for 15 min and the supernatant was filtered. Then filtered supernatant tissue suspension of dermis and epidermis was further extracted with methanol and filtered. The filtrate was analyzed by serial dilutions if necessary using UV-Visible spectrophotometry.

Drug Excipients Compatibility Studies

Fourier Transform Infrared Spectroscopy- Infrared studies were conducted to find out the interaction between the drug and other components in the formulation. FT-IR spectra of mupirocin, optimal microemulsions along with optimal gel formulation were recorded. Samples are prepared by KBr pellet method and subjected to infrared radiations. The scanning range for FT-IR studies were 4000 to 400 cm^{-1}

RESULT AND DISCUSSION

Saturation solubility studies

From the solubility studies rose oil, lavender oil and teatree oil were selected as oil phases based on the drug solubility and Tween 80 and ethanol were selected as surfactant and co-surfactant respectively (Figure 1). Tween 80 was chosen as surfactant because of its high hydrophilic nature (HLB = 15) and good emulsion forming capacity. Previous reports indicated that the superior dermal flux appeared mainly due to large solubilizing capacity of microemulsions, which leads to larger concentration gradient towards the skin.

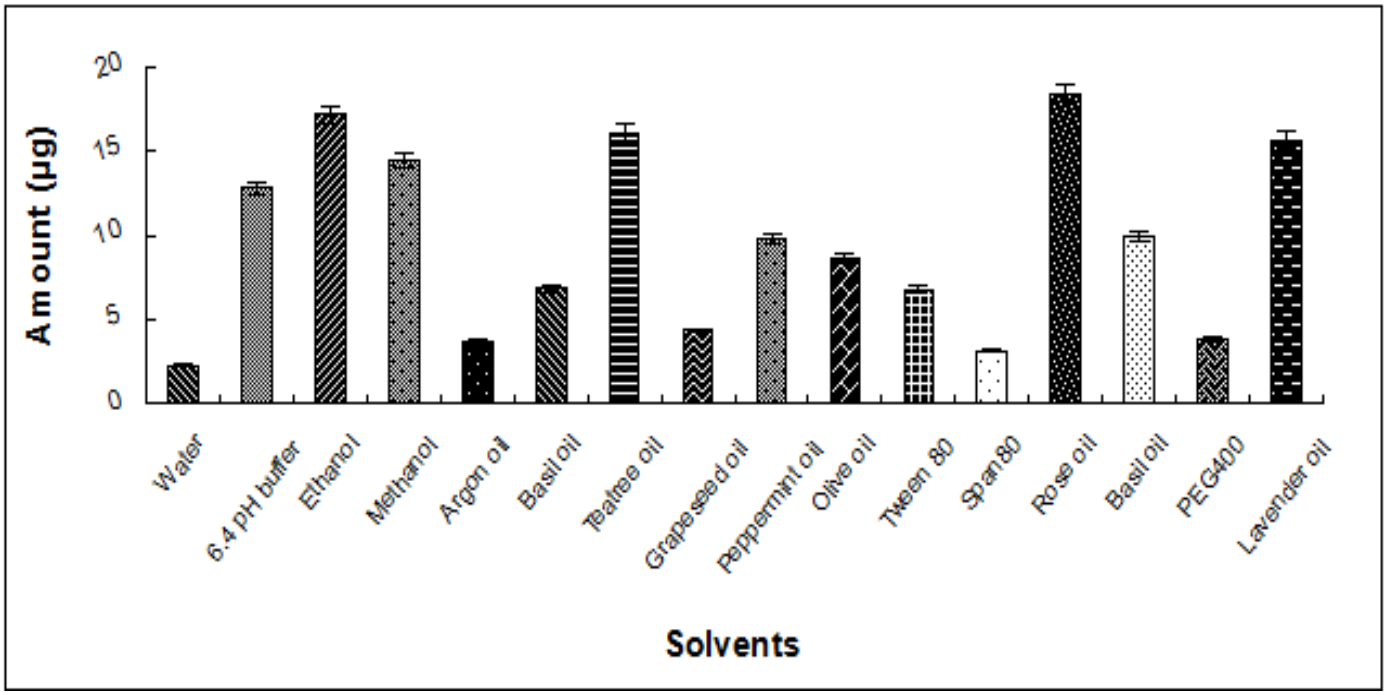
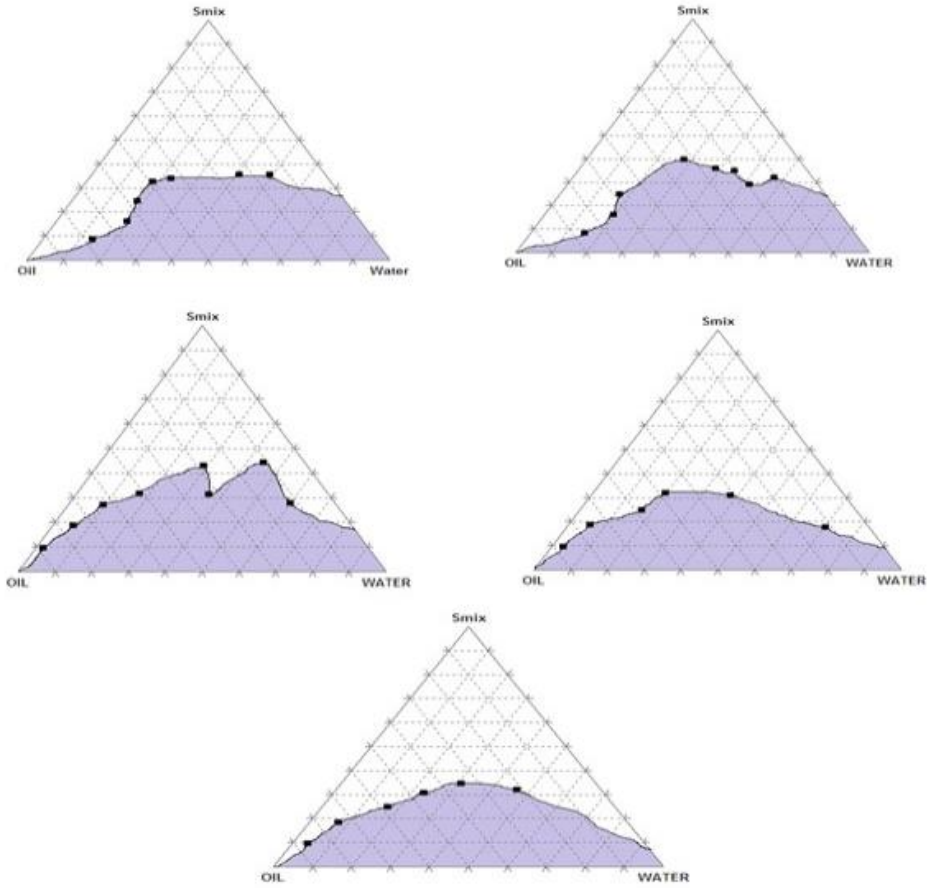


Fig. 1: Solubility of Mupirocin in various Solvents

Construction of Pseudo-Ternary Phase Diagram

Microemulsions were prepared using rose oil, lavender oil and tea tree oil as oil phase, a surfactant Tween 80 and ethanol as co-surfactant and distilled water as aqueous phase (Table 1).

Fig.2 represents pseudo ternary phase diagrams of lavender oil with various ratios of tween80 and ethanol, based on the maximum microemulsion zone obtained; lavender oil was selected for further studies.



a) S:Co = 1:1 ratio, b) S:Co = 1:2 ratio, c) S:Co = 1:3 ratio,

d) S:Co = 2:1 ratio, e) S:Co = 3:1 ratio

Fig. 2: Pseudo-ternary Phase Diagrams of Lavender oil Microemulsion Formulations

Development of Microemulsion Formulations

From the pseudo-ternary phase diagrams formulations LMF8 represented maximum microemulsion zone hence selected for further studies. Formulations LMF1 to LMF7 were clear/transparent but the microemulsion zone obtained was less, whereas LMF9 showed milky appearance after 24h so these formulations were not considered for further studies.

Table 1: Composition of Lavender Oil Microemulsion Formulations

Formulation code	Smix (1:3) (% w/w)	Oil (%w/w)	Water (%w/w)	Drug (mg)
LMF1	9.1	86.5	4.4	20
LMF2	18.1	72.7	9.2	20
LMF3	26.7	62.5	10.8	20
LMF4	33.3	50	16.7	20
LMF5	39.6	39.6	20.7	20
LMF6	41.0	27.39	31.6	20
LMF7	44.8	19.23	35.9	20
LMF8	28.57	7.1	64.35	20
LMF9	23.1	2.57	74.32	20

Characterization of Microemulsion

Measurement of pH and viscosity

Developed formulation LMF8 showed pH which was near to skin pH indicating acceptable range and the results found to be in the range of 5.6±0.2 to 6.8±0.1 as shown in tables 2. Viscosity of the formulation was in the range of 7.5±0.2 to 14.9±0.2.

Determination of drug content

Drug content of all the microemulsion formulation was determined and found to be within the limits as shown in table2.

Thermodynamic stability studies

During heating and cooling cycles and dilution test, formulations LMF1, LMF2 have shown precipitation and hence they were excluded and remaining formulations were found stable, hence they were further evaluated by centrifugation cycle at 3500 rpm. All the formulations (LMF3-F9) were centrifuged at 3500 rpm. During centrifugation LMF9 has shown instability so it was eliminated and remaining formulations were evaluated for further studies. The overall stability of microemulsion formulation is shown in table 2.

Table 2: Physicochemical Characterization of Lavender Oil Microemulsion Formulations

Formulation code	Viscosity (cps)	pH measurement	Drug content (%)	Stability
LMF1	7.8±0.6	5.8±0.5	92.1±0.7	Stable
LMF2	8.4±0.5	5.9±1.2	89.6±0.5	Unstable
LMF3	8.6±1.1	6.4±1.4	89.8±0.6	Stable
LMF4	7.8±1.4	6.8±0.6	95.4±0.9	Stable
LMF5	10.4±0.5	6.8±0.8	91.7±1.1	Stable
LMF6	11.4±0.6	5.9±0.4	79.1±1.2	Stable
LMF7	9.6±0.9	6.2±0.2	94.2±0.4	Stable
LMF8	9.4±0.8	6.4±0.4	96.4±0.2	Stable
LMF9	9.8±1.1	5.8±1.1	91.3±1.6	Unstable

Measurement of droplet size and zeta potential

The globule size plays a significant role in the micro emulsion. The mean size of the globules is shown in table 3. As the concentration of surfactant mixture increased the globule size decreased. Polydispersity indicates the uniformity of droplet size within the formulation. The higher the polydispersity, the lower the uniformity of the droplet size in the formulation. The PDI was within the acceptable limit for formulation LMF8 with size high zeta potential.

Table 3: Globule Size Analysis

Formulation	Droplet size (nm)	Zeta potential	PDI
LMF8	175.4	- 43.5	0.171

***In vitro* drug diffusion studies**

In vitro release studies were performed for mupirocin loaded microemulsions by using dialysis method. It is observed from the studies (Figure 3) that selected formulations LMF8 showed more than 93 % drug diffusion for 6h. This indicates that the diffusion was increased by the use of surfactant mixture at highest ratio.

Formulation Development of Microemulsion Based Gel

The optimal microemulsion formulations LMF8, was incorporated into gel base by continuous stirring until a homogenous microemulsion based gel (LMG8) was formed. The pure drug solution of same concentration (control) was also incorporated into gel base to form control gel for comparative analysis with the optimal formulation.

Characterization of Microemulsion Based Gel

Physical Appearance

The prepared LMG8 was inspected for colour, appearance and consistency, the gel showed smooth homogenous texture and glossy appearance.

Determination of drug content

Drug content was determined in the optimal formulation and found to be within the range 96.8±0.3% for LMG8.

Rheological study and pH measurement

Rheological behavior of the microemulsion based gel system and the pH of the LMG8 was in the range of skin pH which was acceptable.

Spreadability coefficient

The optimal gel formulations (LMG8) showed good Spreadability in less than 1minute. The data obtained for characterization of microemulsion based gel is given in table 4.

Table 4: Characterization of Microemulsion based Gel Formulations

Formulation *	Physical Appearance	Viscosity (cps)	pH measurement	Drug content (mg)	Spreadability (sec)
Control Carbopol 934 gel	Translucent	47,865±1.6	6.2 ± 0.5	88.6 ± 0.3%	50 sec
LMG8	White	45,678±1.4	6.5 ± 0.4	96.8± 0.3%	45 sec

Average of n=3 ±S.D. indicates micro emulsion based gel

***In vitro* Diffusion Studies of Microemulsion based Gel**

In vitro drug release studies were performed for mupirocin loaded microemulsion (LMF8), microemulsion based gel (LMG8) and Control gel (CG).The results are graphically represented in Fig.3. From the studies it was observed that LMG8 showed 72±1.4% drug diffusion in 6h and the percent of drug diffused for control gel was only 31.93±0.6%. This is evident from the studies that a microemulsion improves the permeability of drug through dialysis membrane.

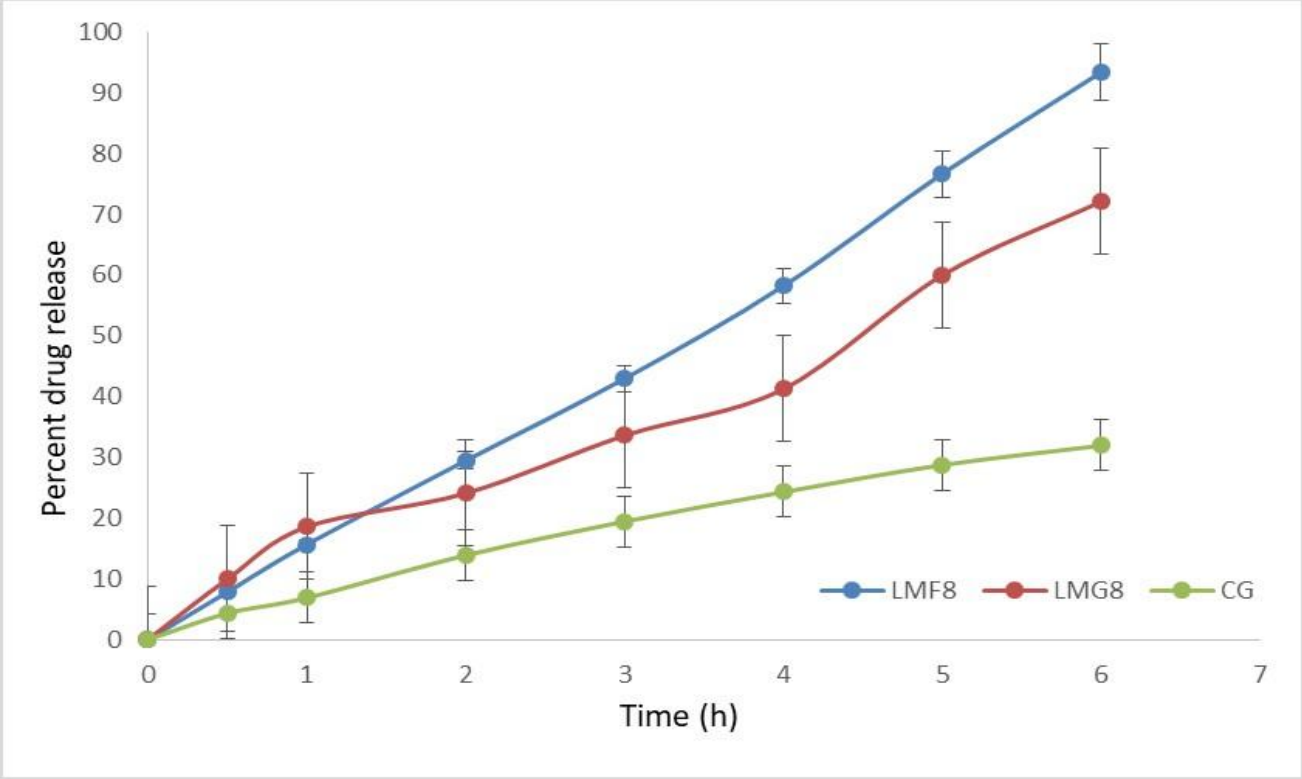


Fig. 3: *In- vitro* Diffusion Studies of Gel Formulations

***Ex vivo* skin permeation studies**

From the *ex vivo* skin permeation studies of optimal gel formulations (LMG8), control gel (CG) and optimal microemulsion (LMF8) it was observed that about 49.99 % of drug was diffused through porcine skin for LMG8 whereas, control gel showed only 15.56% of drug diffusion. From the data it is clearly evident that a microemulsion improves the diffusion of drug through the biological membranes.

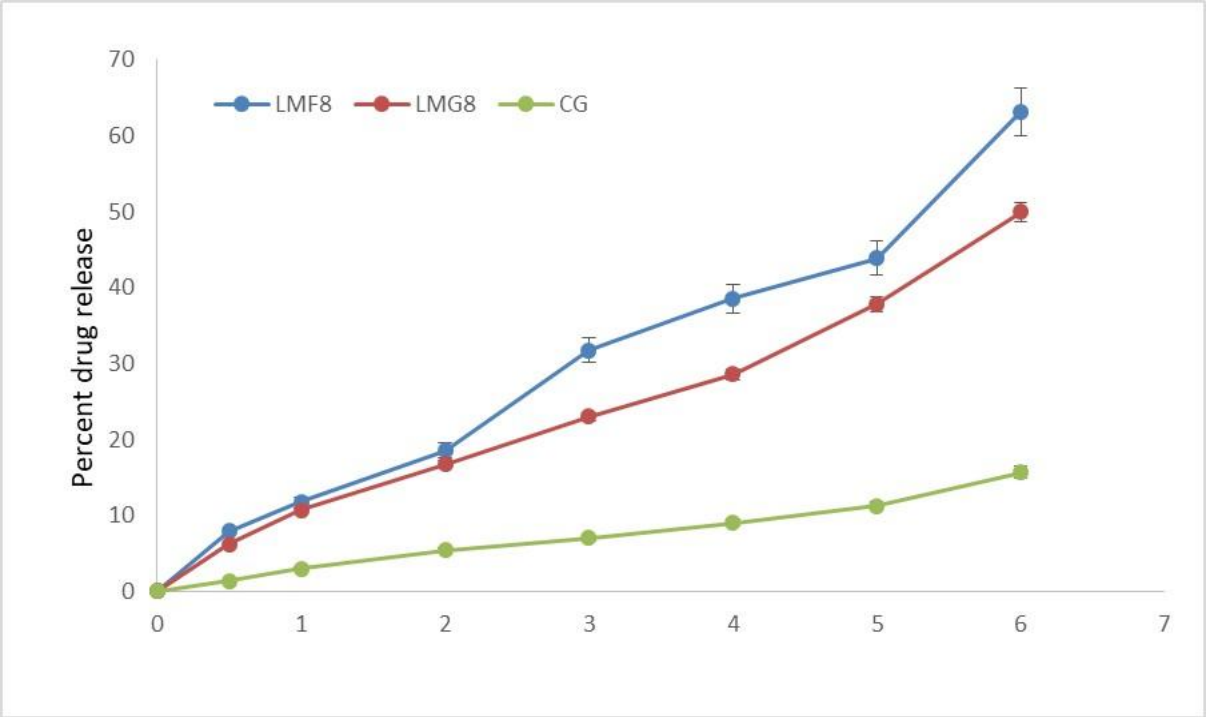


Fig. 4: *Ex vivo* Diffusion Profile of Gel Formulations

Drug retention studies

After separation of skin layers by heat method the amount of drug retained in epidermis and dermis layers was determined for optimal gel formulation (LMG8) and Control gel (CG). The concentration of drug in the washings of skin surface was found to be negligible. Results clearly indicate that the retention in dermis layer was considerably higher in case of LMG8 as compared to control gel. The data clearly indicates that microemulsion can permeate through deeper layers of skin and retains in high concentration which can prove to be effective in treatment of skin burns.

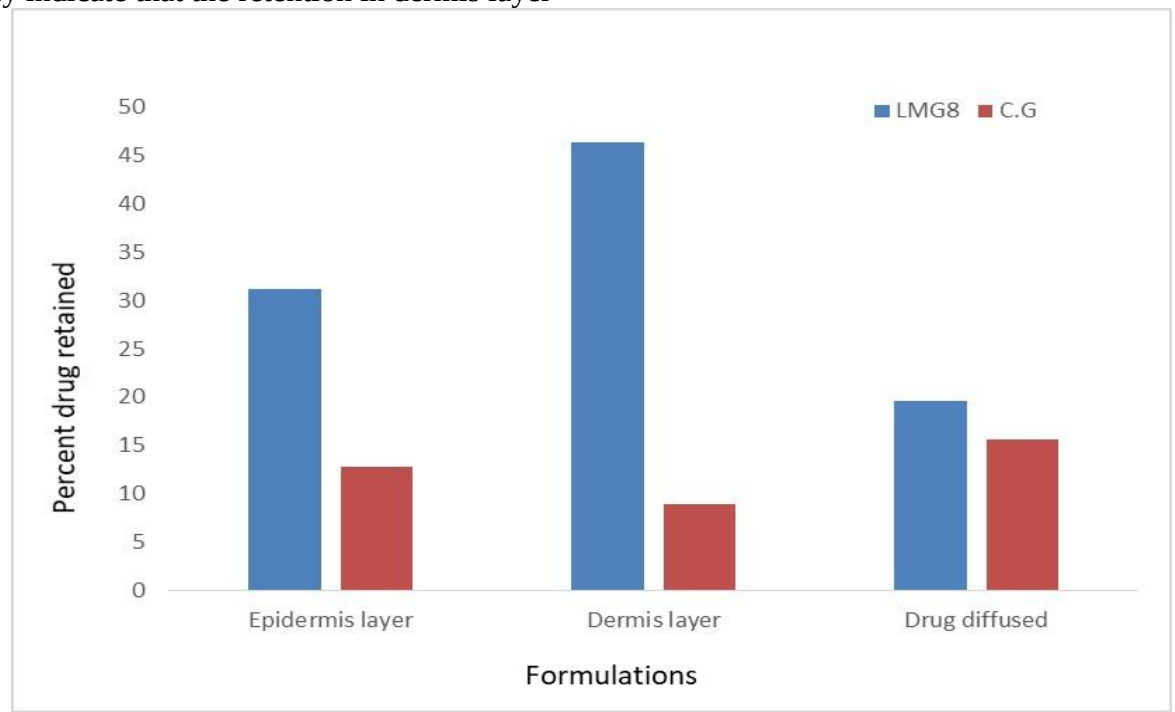


Fig.5: Retention of Drug in Skin Layers for Gel Formulations

Drug Excipients Compatibility Studies

Fourier Transform Infrared Spectroscopy

Infrared studies were conducted to rule out the interaction among the drug, surfactant, co-surfactant and various oils used in the formulation. Compatibility studies of pure drug and the excipients were carried out prior to the formulation development. I.R spectra of pure drug, oil and surfactant mixture, microemulsions, optimal microemulsion based gel formulations were obtained, which are shown in figure 6. All the characteristic peaks mupirocin were present in the formulations spectra thus indicating compatibility between drug and excipients. It shows that there was no significant change in the chemical integrity of the drug and the functional groups present in mupiorocin, microemulsion formulations and microemulsion based gel formulations shown in figure 6.

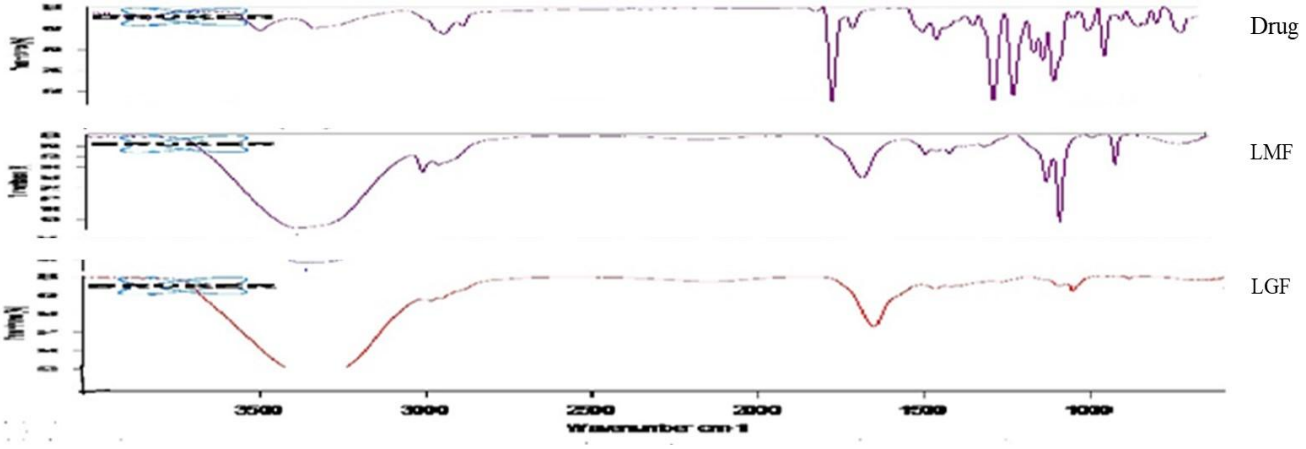


Fig. 6: FT-IR Studies of Drug, Formulation (LMF) and Gel Formulations (LGF)

CONCLUSION

From the results obtained in the studies it can be concluded that the developed mupirocin microemulsion based gel has better drug penetration and retention in skin layers and can be efficiently used to treatment of burns and burn wounds with *staphylococcus aureus* infection.

ACKNOWLEDGEMENTS

Authors thankful to Cipla Ltd. Mumbai, India, for providing gift sample of 'Mupirocin' for carrying out our research project.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

REFERENCES

1. Araya H., Tomita M., Hayashi M. (2005). The novel formulation design of O/W microemulsion for improving the gastrointestinal absorption of poorly water soluble compounds. International Journal of Pharmaceutics, 305, 61–74.

2. Kuljit S., Bhatia, Gao S., Thomas P., Freeman, Singh, J. (1997). Effect of penetration enhancers and iontophoresis on the ultrastructure and cholicystokinin-8 permeability through porcine skin. Journal of Pharmaceutical Sciences, 86 (9), 1011-1015.

3. Danielsson I., Lindman B. (1981).The definition of microemulsion. Colloids and Surfaces,3,391–392.

4. Constantinides P.P. (1995). Lipid microemulsions for improving drug dissolution and oral absorption: physical and biopharmaceutical aspects. Pharmaceutical Research, 12, 1561- 1572.

5. Gasco M.R. (1997). Microemulsions in the pharmaceutical field: perspectives and applications in; Industrial Applications of Microemulsions, Marcel Dekker Inc., New York, 97-122.

6. Gasco M., Gallarate M., Trotta M., Gremmo E., Chiappero O. (1989).Microemulsions as topical delivery vehicles: ocular administration of timolol. Journal of Pharmaceutical and Biomedical Analalysis, 7(4), 433-439.

7. Herndon D. (2002). Treatment of infections in burns. In: Total burn care 2nd Edition. London WB Saunders.

8. Judith E. (2010). Emergency Medicine: A Comprehensive Study Guide (Emergency Medicine (Tintinalli)). New York. 1374-1386.

9. Hsiu O., Huang M., Chen L.L, Angel H., Chen K.TSheu M.T. (1998). The percutaneous delivery of prostaglandin E and its alkyl esters by microemulsions. Chinese Pharmaceutical Journal, 50, 257–266.
10. Hadgraft. J. Lane M.E. (2011). Skin: The ultimate interface. Physical Chemistry Chemical Physics, 13, 5215-5222.
11. Kantaria S., Rees G.D., Lawrence M.J. (2003). Formulation of electrically conducting microemulsion based organogels. International Journal of Pharmaceutics, 250, 65-83.
12. Kawakami K., Yoshikawa T., Moroto Y., Kanaoka E., Takahashi K., Nishihara Y., Masuda K. (2002).Microemulsion formulation for enhanced absorption of poorly soluble drugs prescription design. Journal of Controlled Release, 81, 65–74.
13. Kawakami K., Yoshikawa T., Hayashi T., Nishihara Y., Masuda K. (2002).Microemulsion formulation for enhanced absorption of poorly soluble drugs II. *In vivo* study. Journalof Controlled Release, 81, 75–82.
14. Kreilgaard M. (2002). Influence of microemulsions on cutaneous drug delivery. Advanced Drug Delivery Reviews, 54(1), S77–S98.
15. Laithy H.M. E., Shaboury K. M. E. (2002). The development of cutinalipogel and gel microemulsion for topical administration of fluconazole. AAPS Pharmaceutical Science and Technology, 3(4), 1-9.
16. Morgan E., Bledsoe S., Barker J. (2000). Ambulatory Managements of Burns. American Family Physician, 62(9), 2015-2026.
17. Santos P., Watkinson A.C, Hadgraft J, Lane M.E. (2002). Application of Microemulsions in Dermal and Transdermal Drug Delivery. Skin Pharmacology 243-54.
18. Senthil K. K., Dhachinamoorthi D, Saravanan R. (2011).Microemulsions as Carrier for Novel Drug Delivery: A Review. International Journal of Pharmaceutical Science Review and Research, 10, 37-45.
19. Strock L.L. (1990). Topical Bactroban (Mupirocin): Efficacy in treating burn wounds infected with methicillin-resistant staphylococci. (Journal of Burn Care Rehabilitation, 11(5), 454-459.
20. Talegaonkar S., Azeem A., Ahmad F.J., Khar R. K., Pathan S. A., Khan Z. I. (2008)Microemulsions: a novel approach to enhanced drug delivery. Recent Patents on Drug Delivery and Formulations, 2, 238-257.
21. Tenjarla S. (1999).Microemulsions: an overview and pharmaceutical applications. Critical Review and Therapeutic Carrier System, 16, 461-521.